THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 64:253–257, 1975

Vol. 64, No. 4
Printed in U.S.A.

STUDIES ON THE MODIFYING EFFECT OF ULTRAVIOLET RADIATION ON CHEMICAL SKIN CARCINOGENESIS

FREJ STENBÄCK, M.D.

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska

The effect of ultraviolet light on chemically-induced skin carcinogenesis in mice was studied. Ultraviolet (UV) irradiation (5.5×10^7 ergs/cm^2) 24 hr before repeated applications of 7,12-dimethylbenz[a]anthracene (DMBA) resulted in an increased tumor yield, while irradiation 1 hr after painting caused a decrease. UV irradiation prior to a single application of 3,4-benzo[a]pyrene as an initiating agent, followed by repeated applications of croton oil, increased the neoplastic response. The enhancing effect of a low, nonulcerating dose of UV light 2.8×10^6 ergs/cm^2 on initiation by DMBA in two-stage skin carcinogenesis was also significant.

Reports on the effects of combined ultraviolet (UV) irradiation, visible light, and polycyclic hydrocarbon treatment on animal skin have presented conflicting results. Acceleration of tumorigenic effect has been reported [1–6], as well as inhibition [7,8], and no effect [9–11]. In previous studies in our laboratory, a single application of UV light, given before initiation with 7,12-dimethylbenz[a]anthracene (DMBA), had an enhancing effect on skin tumor induction, while irradiation after initiation caused a decrease in tumor yield [12]. This divergent effect was also obvious when UV light was given once, before repeated applications of DMBA, in which a significant increase in tumor numbers was seen. Conversely, a decrease in the number of tumors occurred when UV light was given after each carcinogen application in a repeated treatment experiment [13].

To more fully evaluate these results, we performed a series of experiments using the carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) and 3,4-benzo[a]pyrene (B[a]P) in a dose and mode of administration (subcutaneous vs topical) differing from previous experiments. In addition, the sequence and interval between UV treatment and carcinogen applications were varied.

MATERIALS AND METHODS

Groups of 40 8-week-old female Swiss mice from the Eppley colony were used. They were housed 10 per plastic cage, fed Rockland diet pellets, and given tap water ad libitum. The light source was a Westinghouse FS-40-T-12 sunlamp emitting an energy of 1.7×10^7 ergs/cm^2/hr at a distance of 37 cm, 55% in the 275 to 315 nm range. Light energy was measured with an International Light IL335 exposure meter. DMBA and B[a]P were applied with a precision pipette on the back skin between the flanks in a volume of 0.02 cc per dose. The skin was shaved 2 hr before UV treatment using electrical clippers and avoiding possible superficial skin injury. The chemicals were checked for purity by thin-layer chromatography and dissolved in twice-distilled acetone (Fisher Scientific Co., Rocklawn, Ill.).

In experiment A (Tab. I), group 1 mice were treated with 12 μg of DMBA (Aldrich Chemical Co., Milwaukee, Wisc.) in acetone twice weekly for 8 weeks, and group 2 received UV light (5.5×10^7 ergs/cm^2) for 3 hr twice weekly for 8 weeks. Group 3 received UV light (5.5×10^7 ergs/cm^2) 24 hr before each carcinogen treatment (DMBA, 12 μg twice a week) for 8 weeks; group 4 received UV light (5.5×10^7 ergs/cm^2) 1 hr before each DMBA application (12 μg twice a week) for 8 weeks; and group 5 received UV light (5.5×10^7 ergs/cm^2) 1 hr after each DMBA application (12 μg in acetone twice a week) for 8 weeks.

In experiment B, group 1 received a single exposure of UV light (5.5×10^7 ergs/cm^2); group 2 received B[a]P (100 μg in acetone) once; and group 3 received croton oil (0.02 cc of a 2.5% solution) twice a week for 30 weeks on the back skin. Group 4 received B[a]P (100 μg) once, followed 10 days later by croton oil (0.02 cc of a 2.5% solution) for 30 weeks. Group 5 received one application of UV light (5.5×10^7 ergs/cm^2) 1 hr before the B[a]P and croton oil. Group 6 received 50 μg of DMBA in acetone applied topically to the back. Group 7 received DMBA (50 μg) and 10 days later croton oil (0.02 cc of a 2.5% solution) twice a week for 30 weeks; group 8 received UV light (2.8×10^6 ergs/cm^2) once and 1 hr later DMBA (50 μg); and group 9, the same treatment followed 10 days later by twice-weekly applications of 2.5% croton oil for 30 weeks. Group 10 received 200 μg of DMBA subcutaneously, and in group 11 this was followed 10 days later by treatment with a 2.5% solution of croton oil in acetone twice weekly for 30 weeks. In group 12, UV light (5.5×10^7 ergs/cm^2) was given once 1 hr before a single injection with B[a]P (100 μg) followed 10 days later by a 30-week schedule of croton oil, 2.5% solution twice a week. Details of the experiments are presented in Table II.

Animals were checked weekly, and tumors, including spontaneous regressions, recorded. All mice were killed 30 weeks after the start of the experiment and were completely autopsied. Sections from all skin lesions (except

Manuscript received May 28, 1974; in revised form November 14, 1974; accepted for publication November 18, 1974.

This work was supported by USPHS Contract PH 43 NCI E 68 959 from the National Cancer Institute.

Reprint requests to: Dr. F. Stenbäck, The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, Nebraska 68105.

TABLE I. *Experiment A: Number and types of tumors induced by repeated applications of ultraviolet light and different chemicals*

Group	Treatment	Total no. of animals	Total TBA[a]	Total gross tumors	Number of animals with histologically verified tumors				
					Papillomas	Squamous cell carcinomas	Hemangiomas	Fibromas	Fibrosarcomas
1	DMBA alone	40	20	28	10	2	—	—	—
2	UV alone	40	10	12	3	2	—	—	2
3	UV 24 hr before DMBA	40	31	37	11	4	1	2	2
4	UV 1 hr before DMBA	40	18	18	5	1	1	1	1
5	DMBA 1 hr before UV	40	8	10	4	—	—	—	1

[a] TBA = tumor-bearing animals

TABLE II. *Experiment B: Modifying effect of ultraviolet light on number and tumor types induced by initiation-promotion treatment*

Group	Treatment			Total no. of animals	Total TBA	Total no. of gross tumors	Total no. of animals with histologically verified tumors			
	Pretreatment ergs/cm²	Initiation	Promotion				Papillomas	Squamous cell carcinomas	Keratoacanthomas	Fibrosarcomas
1	UV 5.5×10^7	—	—	40	—	—	—	—	—	—
2	—	B[a]P 100 μg	—	40	—	—	—	—	—	—
3	—	—	Croton oil 2×/week	40	—	—	—	—	—	—
4	—	B[a]P 100 μg	Croton oil 2×/week	40	4	4	2	—	—	—
5	UV 5.5×10^7	B[a]P 100 μg	Croton oil 2×/week	40	9	14	7	—	—	—
6	—	DMBA 50 μg	—	40	—	—	—	—	—	—
7	—	DMBA 50 μg	Croton oil 2×/week	40	22	28	19	0	1	—
8	UV 2.2×10^6	DMBA 50 μg	—	40	—	—	—	—	—	—
9	UV 2.8×10^6	DMBA 50 μg	Croton oil 2×/week	40	20	40	17	2	—	—
10	—	DMBA 200 μg subcut.	—	40	3	—	—	—	—	3
11	—	DMBA 200 μg subcut.	Croton oil 2×/week	40	2	—	—	—	—	2
12	UV 5.5×10^7	DMBA 200 μg subcut.	Croton oil 2×/week	40	2	5	1	1	—	2

for spontaneously regressing tumors) and from organs showing gross abnormalities were studied histologically. Formalin-fixed sections were embedded in paraffin and stained with hematoxylin and eosin and other stains as needed.

RESULTS

Repeated carcinogen treatment (experiment A) induced a large number of tumors (Fig. 1, Tab. I), the number depending on the conditions of the experiment. DMBA alone induced papillomas and squamous cell carcinomas. With UV irradiation only, papillomas, squamous cell carcinomas, and fibrosarcomas were produced. UV treatment 24 hr before DMBA applications increased the total tumor yield to 37 from 29, and after DMBA applications, UV caused a decrease in the tumor yield from 28 to 10, with only 4 papillomas and a fibrosarcoma remaining. UV given 1 hr before DMBA also caused a decline in tumor yield, although in not as significant an amount as in the previous group. Histologically, the tumors were papillomas and squamous cell carcinomas of varying degrees of differentiation, and a few fibrosarcomas, fibromas, and hemangiomas were seen in the UV-treated animals. Tumors which regressed

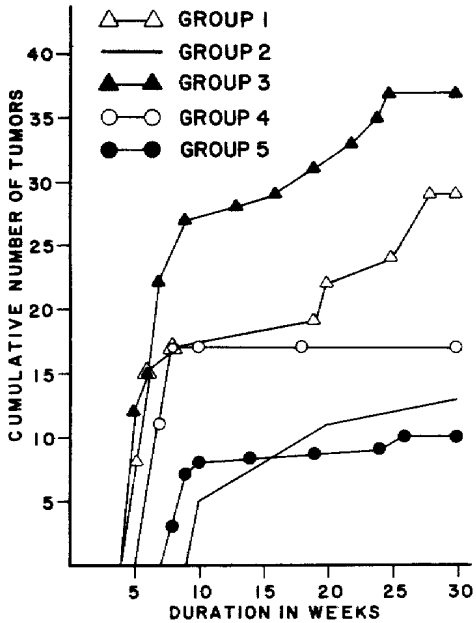

FIG. 1. Experiment A: Number of grossly observed tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) and ultraviolet (UV) light. Group 1 received DMBA only; group 2, UV light only; group 3, UV light 24 hr before DMBA; group 4, UV light 1 hr before DMBA; and group 5, UV light 1 hr after DMBA.

during the experiment were also seen in all groups.

Treatment by the two-stage method (experiment B) almost exclusively induced benign tumors (Fig. 2, Tab. II). UV irradiation, B[a]P, or croton oil treatment alone failed to induce tumors. B[a]P given once and followed by croton oil induced few papillomas. The number increased, however, when the B[a]P initiation was preceded by irradiation with UV light. Histologically, the tumors induced by B[a]P and croton oil were all benign fibropapillomas and acanthopapillomas, with fibrous stalks covered by proliferating squamous epithelium. The irradiation treatment did not alter the histologic character of the tumors, although the stromal response, with fibrosis, vascular proliferation, and elastic degeneration, was more prominent.

Initiation with DMBA, followed by croton oil, produced a higher number of tumors than initiation with B[a]P and croton oil. Morphologically, the tumors were similar to those previously described. DMBA initiation alone produced no tumors. Tumor numbers increased when the DMBA initiation was preceded by small doses of UV irradiation, such as 2.8×10^6 ergs/cm^2. This dose level was devoid of such initial destructive effects as ulceration, fibrosis, and scarring associated with the higher doses of UV light. Most tumors, however, regressed during the experiment and were not available for morphologic analysis. A small number of well-differentiated squamous cell carcinomas invading the dermal tissues as well as a keratoacanthoma composed of proliferating squamous cells on a cup-shaped base were seen.

A single subcutaneous injection of DMBA induced a few fibrosarcomas. Tumorigenesis in this case was not affected by croton oil treatments; however, a small number of epithelial tumors were seen in animals which also received a single dose of UV light prior to initiation.

DISCUSSION

Early studies with combined UV light and tar applications reported increased cancer formation [1,14]. However, these results were not confirmed when pure hydrocarbons were used [11,13,15,16]. Clark [4] reported an increased tumor yield with small doses of UV light on carcinogen-induced tumor formation, but a decrease when large doses of UV light were used. In studies on the effect of visible light on UV-induced skin tumor formation, both retardation [17] and enhancement [18] were found.

In the present study a significant decrease in tumor yield resulted when UV light was given 1 hr after DMBA applications (experiment A, group 5). A probable explanation for this phenomenon is that a photochemical deactivation of the carcinogen [19,20] takes place and influences the carcinogenic effect on the skin [21]. Several early investigators [22–24] showed that UV irradiation caused oxide formation, with DMBA more easily photo-oxidized than B[a]P. Engelbreth-Holm and Iversen [20] reported that the photo-oxide of DMBA was less carcinogenic than the parent compound, when applied topically to mice. Recently, Davies et

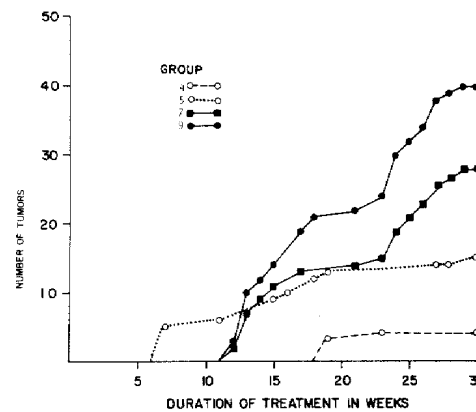

FIG. 2. Experiment B: Number of grossly observed tumors induced by the initiation-promotion method. Group 4 received 3,4-benzo[a]pyrene (B[a]P) once followed by croton oil 2×/week; group 5, ultraviolet (UV) light once 24 hr before B[a]P, followed by croton oil 2×/week; group 7 received 7,12-dimethylbenz[a]anthracene (DMBA) once, followed by croton oil 2×/week; and group 9, UV light once 24 hr before DMBA, followed by croton oil 2×/week.

al [25] reported that photoproducts of DMBA were minimally carcinogenic and that mice, irradiated immediately after painting, exhibited no response to DMBA or light. UV light also inhibited skin tumorigenesis by such other polycyclic aromatic hydrocarbons as dibenz[a,i]pyrene in studies performed in our laboratory (unpublished observations).

Other possibilities, such as a biologic interaction between UV light-induced morphologic abnormalities on DMBA-induced neoplastic progression, must also be taken into account [26]. Argus et al [27] reported an inhibitory effect of x-rays on chemically-induced tumor growth, apparently as a result of the cytotoxic effects of irradiation. It has also been suggested [28] that light may facilitate absorption of carcinogens through the skin, leaving a less effective amount on the surface. Santamaria et al [29,30] considered the inhibition by light a consequence of a photodynamic process leading to severe damage of cellular components, as opposed to the alteration of the carcinogen due to a photosensitized oxidation.

A significant increase in tumor totals was found in the experiments in which UV irradiation was given 24 hr before painting with DMBA. Increased carcinogenic activity of irradiated carcinogens has been reported for B[a]P [30,31] and anthracene [32]. Similarly, in our laboratory, this phenomenon was observed with other carcinogens such as 7-methylbenzanthracene. Contributing to the potential carcinogenesis of B[a]P is the fact that the 6-carbon atom-substituted photoproduct is the most reactive [31]. Epstein [6] explained the increase in tumor yield produced by UV light on DMBA-induced skin tumorigenesis as dependent on the repeated initiation of new latent tumor units. Santamaria et al [29,30], in a series of studies on the effects of UV light on B[a]P skin carcinogenesis, considered the accelerated tumor induction by light as due to excitation of 3,4 B[a]P, a factor still compatible with cellular life. Moreover, phototoxicity known to be associated with polycyclic hydrocarbons [7,29,33,34] appeared to enhance carcinogenic activity in mice irradiated 1 to 4 hr after DMBA applications [25]. Since certain polycyclic hydrocarbons are photosensitizers, the addition of light energy to their inherent carcinogenic effect would be expected to accelerate tumor formation [35].

In an initiation–promotion type experiment we have previously shown that the initiating capacity of DMBA is enhanced by prior UV irradiation [12]. As seen here (experiment B), the increased tumor yield caused by a single UV application prior to initiation does not depend on the type or dose of initiating agent. B[a]P and croton oil (group 4) induced a significantly higher number of tumors when preceded by a single UV light treatment (group 5). UV irradiation, in doses as low as 2.8×10^6 ergs/cm^2 before initiation (group 9), also nearly doubled the tumorigenic response compared to tumor induction by DMBA and croton oil alone. In this group, the initial destructive changes, i.e., necrosis, erosion, and inflammation, were not as significant as in animals receiving the larger dose. A possible explanation for this increase in total tumors may be that an enhanced binding of B[a]P to cellular constituents occurs, increasing the potency of B[a]P [31]. Epstein [35] considered the UV light effect on the hair cycle significant. Our results do not support this hypothesis, as the time period between irradiation and initiation is too short for significant changes in the hair cycle to occur. However, the number of regressing tumors was significant in this, as well as in previous studies [12]. The totals indicate that the increased tumor population possibly represents a lower level of neoplastic transformation induced by the addition of UV light or depending on a specific effect on the host or on its immunologic defense mechanisms.

The author wishes to acknowledge the expert technical help of Mrs. Dorothy Schultz and the editorial assistance of Mrs. Mardelle Susman.

REFERENCES

1. Findlay GM: Ultraviolet light and skin cancer. Lancet 2:1070–1073, 1928
2. Maisin J, de Jonghe A: Au sujet de l'action de la lumiere et de l'ozone sur certains corps cancerigenes. Comp Rend Soc Biol 117:111–114, 1934
3. Vles F, de Coulon A, Ugo A: Recherches sur les proprietes physicochimiques des tissues en relation avec l'etat normal ou pathologique de l'organisme. XXI. Influence de l'obscurité et de la lumere sur la cancerisation par le gourdon. Arch Physique Biol 12:255–265, 1935
4. Clark JH: The effect of long-wave ultraviolet radiation on the development of tumors induced by 20 methylcholanthrene. Cancer Res 24:207–211, 1964
5. Epstein JH, Epstein WL: Cocarcinogenic effects of ultraviolet light on DMBA tumor initiation in albino mice. J Invest Dermatol 39:455–460, 1962
6. Epstein JH: Comparison of the carcinogenic and cocarcinogenic effects of ultraviolet light on hairless mice. J Natl Cancer Inst 34:741–745, 1965
7. Doniach I, Mottram JC: On the effect of light upon the incidence of tumors in painted mice. Am J Cancer 39:234–240, 1940
8. Morton JJ, Luce-Clausen EM, Mahoney EB: Visible light and skin tumors induced with benzpyrene in mice. Cancer Res 2:256–260, 1942
9. Kohn-Speyer AC: Effect of ultraviolet radiation on the incidence of tar cancer in mice. Lancet 1:1305–1306, 1929
10. Seelig MG, Cooper ZK: Light and tar cancer. Surg Gynecol Obstet 56:752–761, 1933
11. Tausig J, Cooper ZK, Seelig MG: The effect of light on benzpyrene cancer in mice. Surg Gynecol Obstet 66:989–993, 1938
12. Stenbäck F, Garcia H, Shubik P: Studies on the influence of ultraviolet light on initiation in skin tumorigenesis. J Invest Dermatol 61:101–104, 1973
13. Stenbäck F, Shubik P: Carcinogen-induced skin tumorigenesis in mice: enhancement and inhibition of ultraviolet light. Z Krebsforsch 79:234–240, 1973
14. Teutschlaender O: Bedarf der Teer zur Hautkrebserzeugung ultravioletter Strahlen? Klin Wochenschr 16:1284–1285, 1937
15. Rusch HP, Kline BE, Baumann CA: The nonadditive effect of ultraviolet light and other carcinogenic procedures. Cancer Res 2:183–188, 1942
16. Epstein JH, Sullivan FJ, Epstein WL: The effect of

ultraviolet light on chemical carcinogenesis. J Invest Dermatol 36:73–77, 1961
17. Kelner A, Taft EB: The influence of photoreactivating light on the type and frequency of tumors induced by ultraviolet radiation. Cancer Res 16:860–866, 1956
18. Griffin AC, Dolman VS, Böhlke EB, Bouvart P, Tatum EL: The effect of visible light on the carcinogenicity of ultraviolet light. Cancer Res 15:523–528, 1955
19. Moodie MM, Reid C, Wallick CA: Spectrometric studies of the persistence of fluorescent derivatives of carcinogens in mice. Cancer Res 14:367–371, 1954
20. Engelbreth-Holm J, Iversen S: The effect of ultraviolet irradiation on the carcinogenic potency of certain hydrocarbons. Cancer Res 7:372–378, 1948
21. Miller EC: Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in the epidermal fraction of mouse skin. Cancer Res 11:100–108, 1951
22. Cook JW, Martin RH: Polycyclic aromatic hydrocarbons. XXIV. J Chem Soc (Lond) 1125–1127, 1940
23. Dufraisse C, Gerard M: Axydes organiques disociables et structure anthracenique. Sur l'existence d'un photo-oxyde de l'anthracene: sa decomposition thermique. Comp Rend Acad Sci 201:428–430, 1935
24. Velluz L: Atude de comparison, dans la serie polycyclique, entre l'oxydabilite reversible et le pouvoir carcinogenetique. Comp Rend Acad Sci 206:1514–Photobiol Proc, 347, 1972
25. Davies RE, Dodge HA, Austin WA: Carcinogenicity of DMBA under various light sources. VI. Int Congr Photobiol Proc 347, 1972
26. Epstein JH, Fukuyama K, Dobson R: Ultraviolet carcinogenesis, The Biological Effects of Ultraviolet Radiation. Edited by F Urbach. Oxford, Pergamon, 1969, pp 551–568
27. Argus MF, Kane JF, Sakuntala M, Roy FE: Effect of ionizing radiation on 9,10-dimethyl-1,2-benzanthracene tumorigenesis. Radiat Res 16:37–43, 1962
28. Morton JJ, Mider GB, Luce-Clausen EM, Mahoney EB: The effect of visible light on the development in mice of skin tumors and leukemia induced by carcinogens. Cancer Res 11:559–561, 1951
29. Santamaria L, Giordano G, Alfisi M, Carcione F: Effect of light on 3,4-benzopyrene carcinogenesis. Nature (Lond) 210:824–825, 1966
30. Santamaria L, Giordano G: Effect of long wave ultraviolet radiation on polycyclic hydrocarbon carcinogenesis. The Biological Effects of Ultraviolet Radiation. Edited by F Urbach. Oxford, Pergamon Press, 1969, pp 569–580
31. Cavalieri E, Calvin M: Photochemical coupling of benzo(a)pyrene with 1-methylcytosine: photoenhancement of carcinogenicity. Photochem Photobiol 14:641–653, 1971
32. Heller W: Experimentelle Untersuchungen über den Lichtkrebserzeugung durch Photosensibilisierung. Strahlentherapie 81:529–548, 1950
33. Epstein SS, Burroughs M: Some factors influencing the photodynamic response of paramecium caudatum to 3,4-benzpyrene. Nature (Lond) 193:337–338, 1962
34. Epstein SS, Small M, Falk HL, Mantel N: On the association between photodynamic and carcinogenic activities in polycyclic compounds. Cancer Res 24:855–862, 1964
35. Epstein JH: Ultraviolet light carcinogenesis. Carcinogenesis, Advances in Biology of the Skin. Edited by W. Montagna, RL Dobson. Oxford, Pergamon, 1966, pp 215–226